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## Abstract

Here we present the development of a prototype wound dressing that can detect the infection in wounds. The dressing is made of a hydrated agarose sheet in which a mixture of fluorescent dye containing vesicles and agarose are dispersed within the hydrogel matrix. The release of dye is triggered by the interaction of vesicles with virulence factors, secreted within the biofilms via population-density-dependent quorum sensing, from clinical pathogens of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* (SPE pathogen group). The dressing only activates when in contact with wound biofilm of pathogenic bacteria in infected wounds, but not to the biofilms of non-pathogenic bacteria.

## Introduction

Wound infection is a global problem and approximately 13,000 patients with burns require treatment in hospitals in England and Wales every year.<sup>1,2</sup> Burn wound infection is currently diagnosed by clinical observation and judgement, and standard microbiological culture to identify causative pathogens usually take several days.<sup>3,4</sup> There are evidences that the delayed healing due to persistent infection is closely related to wound biofilm formation.<sup>5,6</sup> If pathogens present, this will cause tissue damage by further colonization, extensive infection and formation of difficult-to-treat biofilm in wounds which inevitably require aggressive antibiotic treatments.<sup>7</sup> Early indication of infection at point of care and ability to rapidly distinguish between infected and non-infected states of wound will help in clinical decision making, prevent over-management by inappropriate use of antibiotics, improve patient outcomes and reduce costs of treatment.

## Methods, Results and Discussion

### 1) Vesicles and Mode of Action of Detection

Vesicles of 200 nm in diameter were made of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), cholesterol and 10,12-tricosadiyonic acid (TCDA) by extrusion.<sup>8</sup> Encapsulated inside vesicles were 50 mmol dm<sup>-3</sup> self-quenched 5,6-carboxyfluorescein in HEPES buffer. Only exotoxins of pathogenic bacteria, such as Phenol-Soluble Modulin (PSM), delta haemolysin and rhamnolipids lysed vesicles and triggered the release of visible dyes (figure 1).<sup>9,10</sup> Expression of such virulence factors by wound pathogens such as *S. aureus* and *P. aeruginosa* was demonstrated by growing them on the blood agar plate (figure 2).

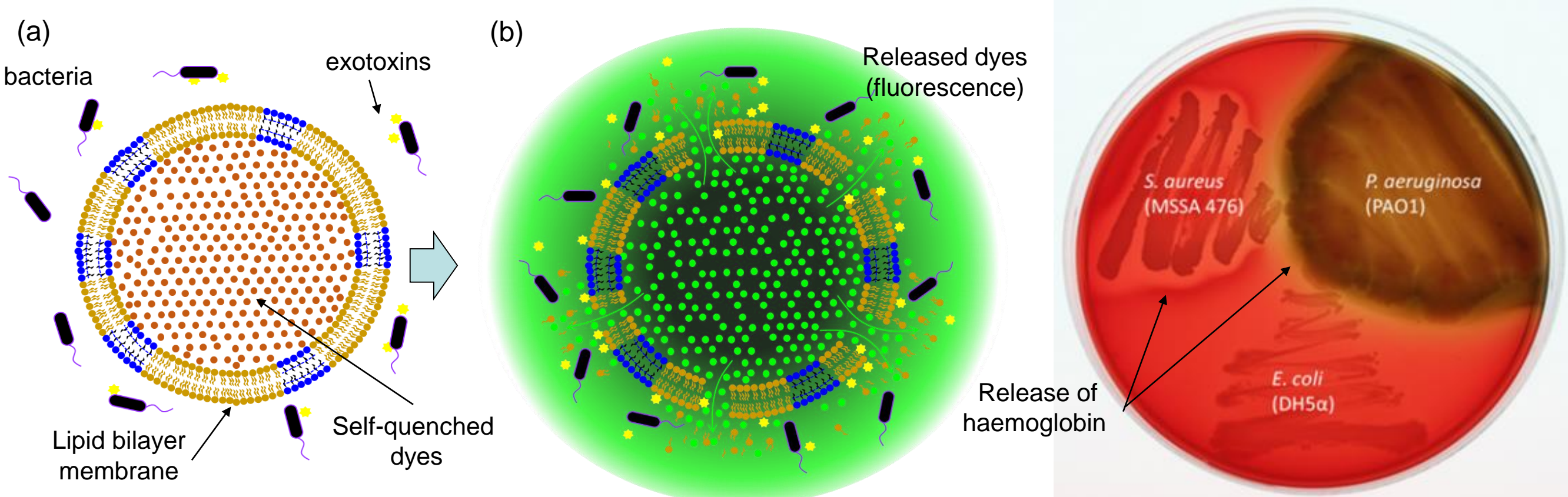


Figure 1. Schematic depiction of mode of action of vesicles against pathogenic bacteria. (a) Pathogens produce toxins that can lyse lipid bilayer membrane of vesicles, and (b) trigger the release of dye which is visible to naked eye.

Figure 2. Exotoxins, such as PSM, delta haemolysin and rhamnolipids of pathogenic *S. aureus* and *P. aeruginosa* lyse erythrocytes in blood agar to release haemoglobin (seen as halo), but not by control *Escherichia coli*.

### 2) Dressing Design and Prototype Development

The vesicles were mixed with molten 0.7% agarose and filled inside the patterned wells of moulded 2% agarose dressing (figure 3a,b).<sup>11</sup> This design allows the diffusion of toxins from infected wound to the dressing, following the fluorescent dyes release from vesicles within hydrogel matrices (figure 3c).

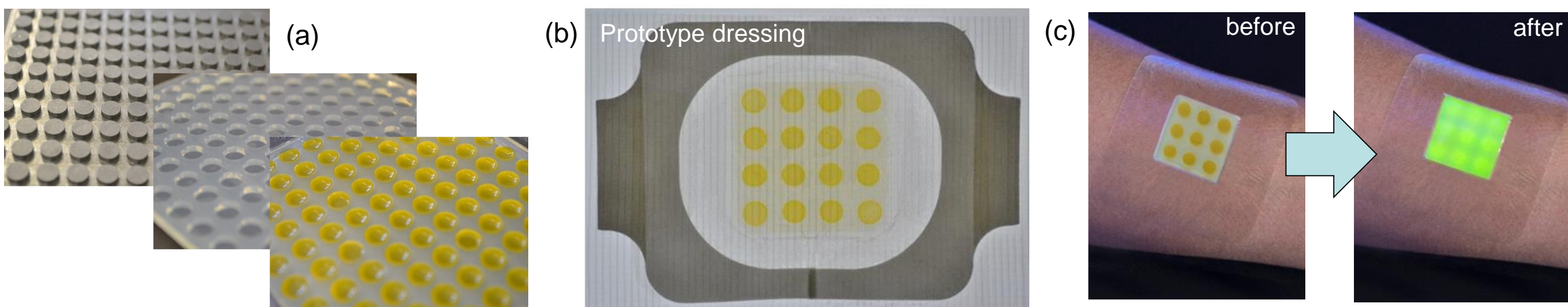


Figure 3. Prototype dressing development (a) Embossed mould is used to make a thin slab of agarose with patterned wells, which are filled with vesicles + agarose, (b) finished prototype, and (c) before and after the activation of dressing with Triton on an arm.

### 3) Clinical Assessment of Prevalent Burn Wound Pathogens

An independent assessment of 55 burn wounds at Queen Victoria Hospital (QVH) at East Grinstead, U.K. provided that more than 75% of "at risk" wounds were colonized by species of pathogenic bacteria, of which above 80% were identified as *S. aureus*, *P. aeruginosa* and *E. faecalis* strains (SPE pathogens) (figure 4)

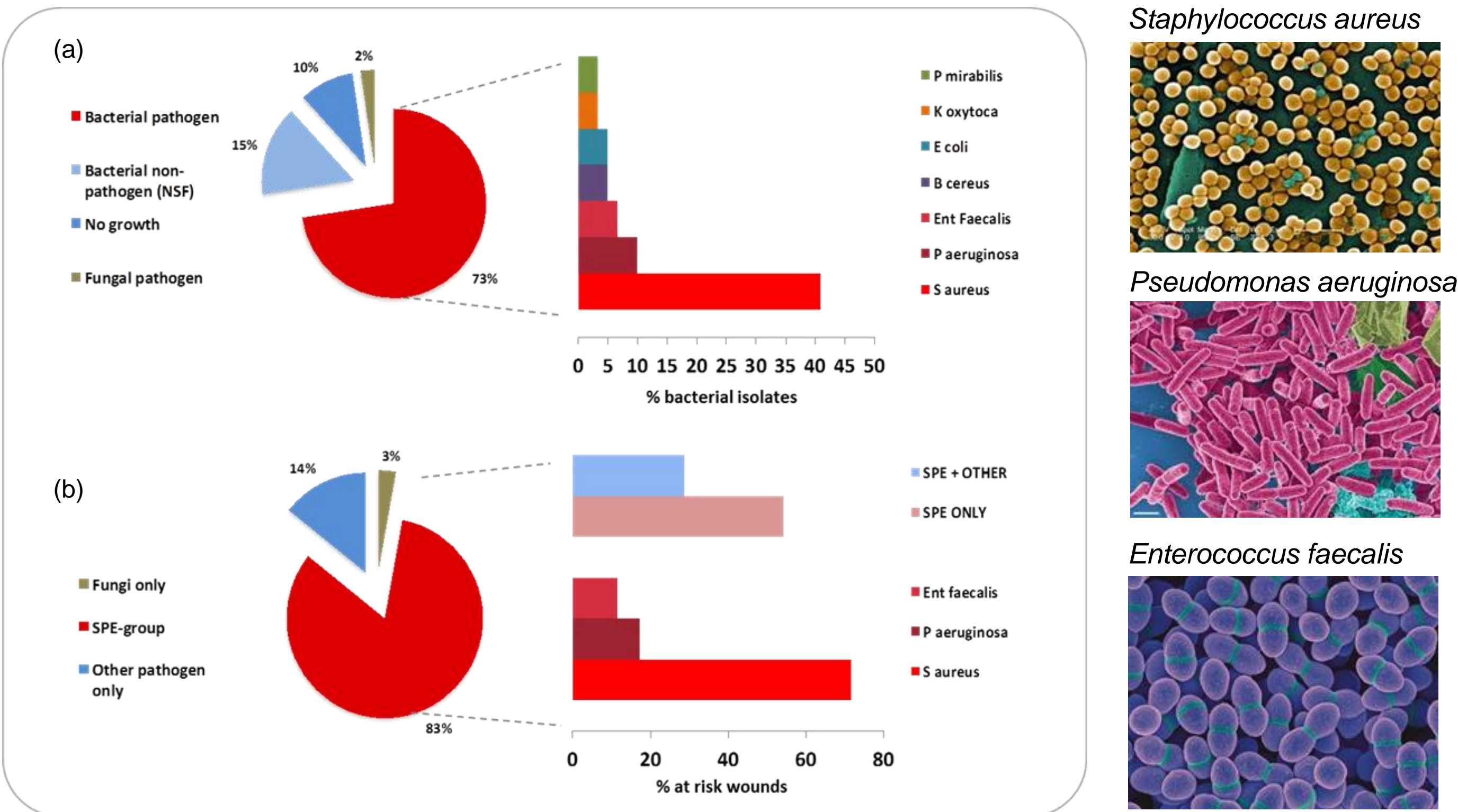


Figure 4. Clinical assessment study of prevalent burn wound pathogens (a) 73% of infected wounds are colonized by pathogenic bacteria while (b) 83% of them are identified *S. aureus*, *P. aeruginosa* and *E. faecalis* with majority of *S. aureus* colonisation.

### 4) In-vitro Colony Wound Biofilm Model

For the evaluation on prototype dressing performance, a model wound biofilm was developed by growing biofilms on a nano-porous polycarbonate membrane (200 nm in diameter) on top of nutrient agar, incubated at 37°C (figure 5a,b).<sup>12</sup> Growth of biofilm was characterized by Scanning Electron Microscope (figure 5c).

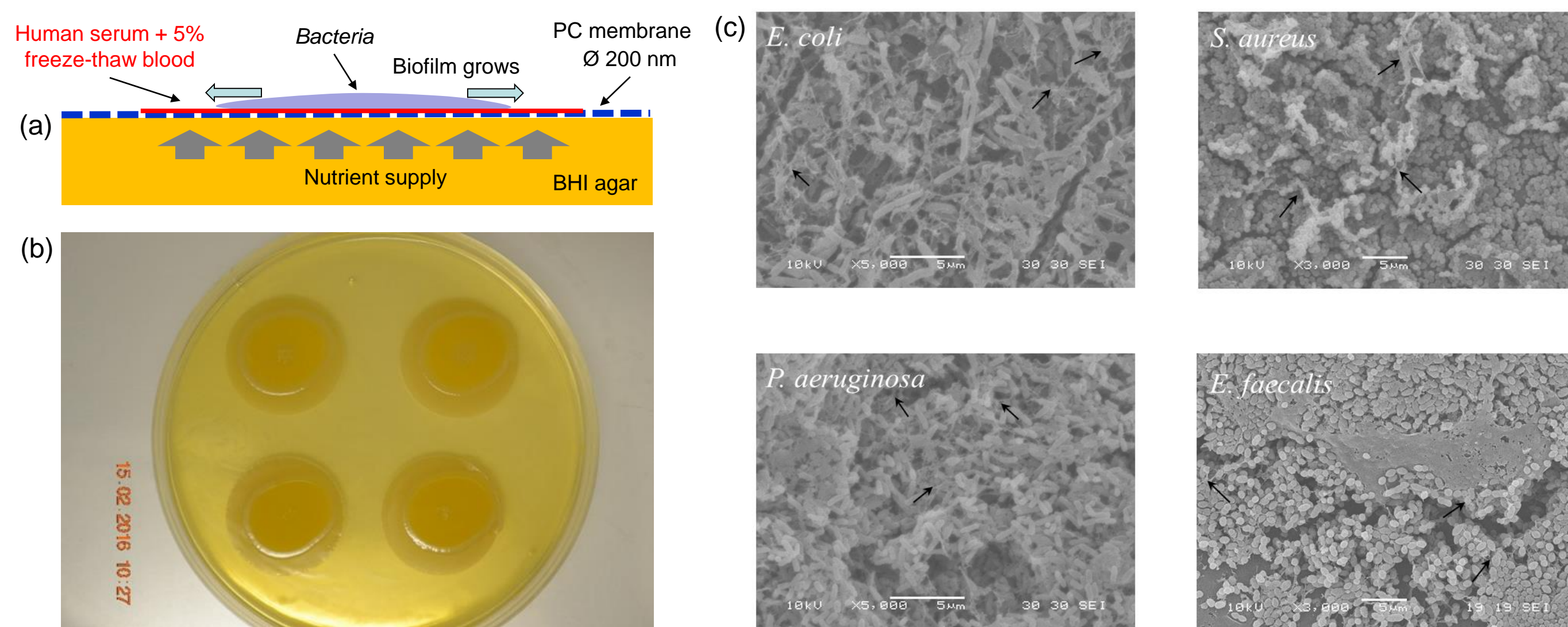


Figure 5. In-vitro colony wound biofilm model (a) schematic depiction of growth of a biofilm by acquiring nutrients through nano-pores, (b) photograph of *S. aureus* biofilms on polycarbonate membranes, and (c) SEM images of biofilms of *E. coli* and selected SPE strains (black arrows indicate the characteristic extracellular polymeric substances (EPS) of a biofilm).

### 5) Dressing Response to In-vitro Model Wound Biofilms

In-vitro colony wound biofilms, produced from burn wound pathogens, *S. aureus* and *P. aeruginosa*, and control *E. coli* were grown for 24, 48 and 72 hours and tested with prototype dressings (figure 6a). Within 5 hours of incubation with biofilms, the fluorescent response was clearly observed in dressings (figure 6b).

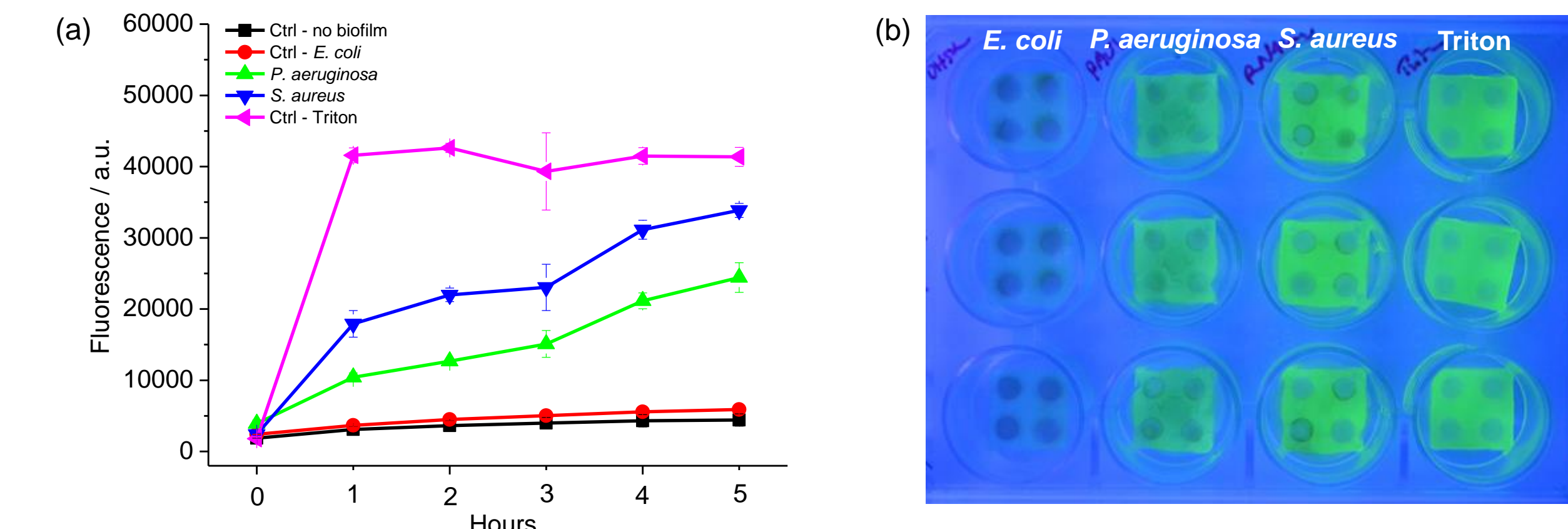


Figure 6. Fluorescent response of prototype dressing to in-vitro model wound biofilms (a) time dependent response of dressings with 24 hour biofilms, and (b) photograph of dressings with biofilms taken under UV (254 nm) light.

### 6) Ex-vivo Porcine Burn Wound Model and Dressing Response

Using organic porcine skins, ex-vivo burn wound, as a more realistic model, was further developed for the evaluation of the dressing performance.<sup>11</sup> Scaled burns were created with a hot metal block on disinfected pig skins (figure 7a) and infected with selected strains of SPE pathogens to establish biofilms allowing only burned tissues as a sole carbon source for infected bacteria (figure 7b). After 24 hours of growth at 37°C, each dressing was placed on the burn wounds and incubated up to 24 hours to test the dressing response (figure 7c). Fluorescent response was observed in all of the dressings except for the control biofilms of *E. coli*.

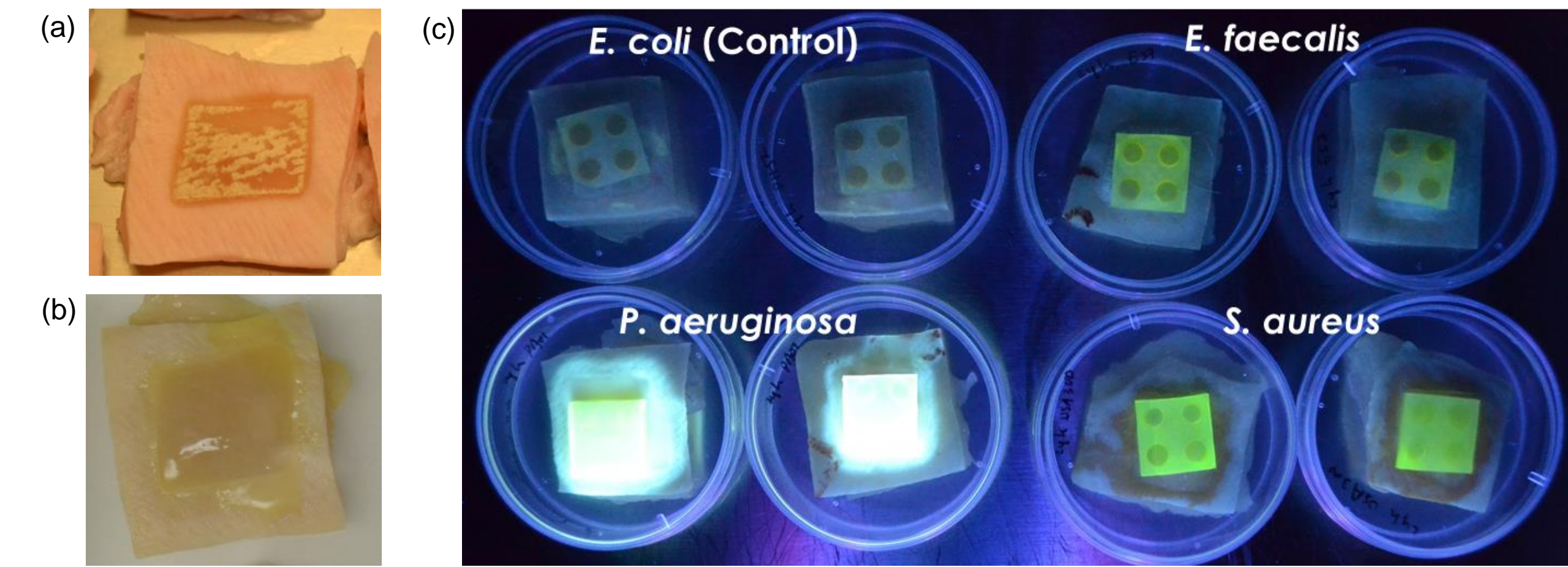


Figure 7. Ex-vivo porcine burn wound model and fluorescent response of prototype dressings (a) scaled burn on a porcine skin, (b) 24 hour biofilm of *P. aeruginosa*, and (c) dressing response to porcine wound biofilms, seen under UV (254 nm) light.

## Conclusion

A prototype intelligent wound dressing is developed and the dressing performance is accessed by testing with in-vitro and ex-vivo porcine wound biofilm models of clinically important *S. aureus*, *P. aeruginosa* and *E. faecalis* (SPE) pathogens. Patterned vesicles in hydrogel dressing are lysed by virulence factors of pathogenic bacteria in model biofilms only and triggered the release of fluorescent dyes which are visible to naked eyes. This study provides proof-of-concept for an advanced infection-detecting dressing for wound care, which could allow the targeted treatment of infections at the bedside and reduce the unnecessary use of antibiotics.

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